Molecular Pathways: Targeting Mechanisms of Asbestos and Erionite Carcinogenesis in Mesothelioma

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Abstract
Malignant mesothelioma is an aggressive malignancy related to asbestos and erionite exposure. AP-1 transcriptional activity and the NF-κB signaling pathway have been linked to mesothelial cell transformation and tumor progression. HGF and c-Met are highly expressed in mesotheliomas. Phosphoinositide 3-kinase, AKT, and the downstream mTOR are involved in cell growth and survival, and they are often found to be activated in mesothelioma. p16INK4a and p14ARF are frequently inactivated in human mesothelioma, and ~50% of mesotheliomas contain the NF2 mutation. Molecular therapies aimed at interfering with these pathways have not improved the dismal prognosis of mesothelioma, except possibly for a small subset of patients who benefit from certain therapies. Recent studies have shown the importance of asbestos-induced inflammation in the initiation and growth of mesothelioma, and HMGB1 and Nalp3 inflammasome have been identified as key initiators of this process. Asbestos induces cell necrosis, causing the release of HMGB1, which in turn may activate Nalp3 inflammasome, a process that is enhanced by asbestos-induced production of reactive oxygen species. HMGB1 and Nalp3 induce proinflammatory responses and lead to interleukin-1β and TNF-α secretion and NF-κB activity, thereby promoting cell survival and tumor growth. Novel strategies that interfere with asbestos- and erionite-mediated inflammation might prevent or delay the onset of mesothelioma in high-risk cohorts, including genetically predisposed individuals, and/or inhibit tumor growth. The very recent discovery that germline BAP1 mutations cause a new cancer syndrome characterized by mesothelioma, uveal melanoma, and melanocytic tumors provides researchers with a novel target for prevention and early detection. Clin Cancer Res; 18(3); 598–604. ©2011 AACR.

Background
"Asbestos" is a nonspecific term that refers to 6 fibrous silicate minerals that are used commercially and are divided into 2 groups, serpentine and amphibole, based on their chemical composition and crystalline structures (1). Among the serpentine minerals, only chrysotile is used commercially. Chrysotile is a hydrated magnesium silicate, and its stoichiometric chemical composition may be given as Mg₃Si₂O₅(OH)₄. Worldwide, more than 95% of the asbestos used commercially is chrysotile (1). The chemical composition of asbestos minerals in the amphibole group can vary widely. The commercially used asbestiform amphiboles are actinolite, tremolite, anthophyllite, amosite, and crocidolite. These minerals are all hydrated silicates and have double tetrahedral chains of Si₈O₂₂ composition that extend along the c-axis. Amphiboles are distinguished from each other by the number of the cations (calcium, iron, magnesium, and sodium) they contain (1). Thus, "asbestos" is a commercial rather than a scientific term. Approximately 396 fibrous minerals are found in nature; 390 of these minerals are not called asbestos, and they are not subject to restrictive regulations because they had not been used commercially at the time regulations to control the use of some mineral fibers were implemented. An unintended consequence of this very confusing nomenclature is that it is often erroneously assumed that only asbestos, and not other mineral fibers, causes cancer. One such naturally occurring fibrous mineral is erionite. Although exposure to erionite is less widespread, it is more potent than asbestos in causing mesothelioma (2, 3).

Exposure to asbestos and other fibrous minerals contributes to asbestosis and some lung cancers, and it is the main cause of mesothelioma, a highly aggressive cancer that arises from mesothelial cells of the pleura, peritoneum, and pericardium, with a median survival of 1 year from diagnosis (1). Currently, mesothelioma causes approximately 3,000 deaths each year in the United States and an additional 5,000 deaths each year in western Europe (1). Despite asbestos abatement efforts, mesothelioma rates have not significantly changed in the United States since 1994, and
they are estimated to increase by 5% to 10% per year in most European countries over the next 25 years (1). The continued use of asbestos in some commercial products within the United States and the difficulty of removing asbestos that is already in place present additional risks for mesothelioma and lung cancer that will persist into the foreseeable future. We can anticipate a dramatic increase in the incidence of mesothelioma and other asbestos-associated malignancies in the third world, particularly in India, where the use of asbestos continues to increase exponentially and few, if any, precautions are taken (4).

Moreover, increased urban development may disturb outcrops of asbestos, erionite, or soil containing other types of carcinogenic mineral fibers, leading to more instances of exposure (1, 2, 5–9). Recent investigations uncovered exposure to erionite in North Dakota, where more than 300 miles of roads, playgrounds, and driveways have been paved, mostly during the past 2 decades, with gravel containing erionite. More erionite exposure is suspected in nearby states. The air concentrations of erionite in cars and school buses transiting on North Dakota roads were found to be equal to or greater than those recorded in some Turkish villages that experienced a 6.3% mortality from mesothelioma (9). A similar problem occurred in New Caledonia, where exposure to antigorite, a type of serpentine mineral fiber that was used as road gravel, led to a mesothelioma epidemic (6).

Because the interval between initial asbestos or erionite exposure and diagnosis ranges from ~25 to 71 years (1, 9), there would be time to implement preventative therapies within exposed cohorts, such as in North Dakota (where most roads were paved with erionite-containing gravel in the recent past), if we could identify the precise mechanisms of asbestos carcinogenesis and develop biomarkers of exposure and mesothelioma to monitor the population at risk.

**Pathogenesis of mesothelioma and mechanisms of asbestos carcinogenesis**

The observation that only a fraction (~5%) of workers exposed to high doses of asbestos for prolonged periods of time developed mesothelioma and the identification of clusters of mesothelioma cases within certain families suggest that genetics influences mineral fiber carcinogenesis (1). Studies of an epidemic of mesothelioma in Cappadocia, Turkey and the United States showed that the risk of developing mesothelioma is transmitted with an autosomal-dominant pattern in certain high-risk families (2). Germline mutations of the BAP1 gene have now been linked to a high incidence of malignant mesothelioma in some U.S. families (10). Individuals with heterozygous BAP1 germline mutations are affected by a novel cancer syndrome characterized by a very high risk of developing mesothelioma, uveal melanoma, and possibly additional cancers (10). Mesothelioma may become dominant in these families upon exposure to asbestos or erionite (10). The identification of BAP1 mutant carriers may be facilitated by the detection of melanocytic nevi, as described by Wiesner and colleagues (11) and confirmed by genetic testing. In addition to familial cases, BAP1 somatic mutations have been identified in 25% of sporadic mesotheliomas (10, 12) and 84% of metastasizing uveal melanomas (13), but they appear to be relatively rare in other cancers (14, 15). BAP1 is a deubiquitinating enzyme that has tumor-suppressor activity (14, 15). BAP1 seems to regulate deubiquitination during the DNA damage response and the cell cycle, thus influencing S-phase progression, cell necrosis, and apoptosis (14, 15). Mutations that abolish the deubiquitinating activity of BAP1 and/or its nuclear localization abolish BAP1 tumor-suppressor activity (15). The exact target of BAP1 remains unclear. Ventii and colleagues (15) proposed that expression of BAP1 induces early exit out of G1, causing an accumulation of DNA damage and cell death. Along these lines, we hypothesize that by influencing DNA damage repair, BAP1 may help prevent environmental carcinogenesis caused by asbestos/erionite or UV light. This would explain the very high incidence of mesothelioma, uveal melanoma, and melanocytic tumors (rather than other nonenvironmentally related cancer types) among BAP1 germ-line mutant carriers.

DNA damage may be caused directly by mechanical interference of asbestos fibers with chromosome segregation during mitosis (16) or, more likely, indirectly by asbestos-related induction of mesothelial cells and macrophages to generate mutagenic reactive oxygen species (ROS) and inducible nitric oxide synthase (iNOS), both of which are mutagenic in vitro (17, 18). The generation of oxidants by macrophages as they attempt to digest asbestos fibers may trigger the activation of several signaling pathways. Two such pathways, mitogen-activated protein kinase (MAPK) signaling and the resulting activator protein 1 (AP-1) transcriptional activity, have been linked to mesothelial cell transformation (19). Other minerals, such as crystalline silica, also elicit ROS and iNOS production from lung macrophages but do not cause mesothelioma (20). Thus, the capacity of asbestos to induce ROS and iNOS is only one of multiple factors that contribute to asbestos carcinogenesis.

In addition to triggering MAPK signaling, asbestos may directly initiate AP-1 activity through EGFR and downstream pathways (19). Similar to EGFR signaling, activation of hepatocyte growth/scatter factor (HGF) and its receptor tyrosine kinase (RTK) c-Met leads to cell proliferation and motility. Both HGF and c-Met are highly expressed in most mesotheliomas, especially those that are SV40-positive (21). HGF activation is mediated through the phosphatidylinositol-3-kinase (PI3K)/MEK5/Fos-related antigen 1 (Fra-1) feedback pathway (19). PI3K, AKT, and the downstream mTOR are involved in cell growth and survival, and they are often found to be activated in mesothelioma (22). P16INK4a and p14ARF are frequently inactivated in mesothelioma (23–25), and ~50% of mesotheliomas contain missense or nonsense mutations in the neurofibromatosis type 2 (NF2) gene (26, 27). It was shown that mice that were homozygous null for the ARF tumor suppressor rapidly developed mesothelioma upon exposure to asbestos and...
that mice that were heterozygous for ARF were also susceptible to mesothelioma and consistently exhibited biallelic inactivation of ARF (28, 29).

**Chronic inflammation and mesothelioma**

Inflammation is the hallmark of asbestos deposition in tissue and contributes to asbestos carcinogenesis (1, 30, 31). The inflammatory infiltrate into tissue areas containing asbestos deposits consists largely of phagocytic macrophages that internalize asbestos and release numerous cytokines and mutagenic ROS (32). Two such cytokines, TNF-α and interleukin (IL)-1β, have been convincingly linked to asbestos-related carcinogenesis (31, 33). Both IL-1β and TNF-α were shown to enhance erionite fiber-induced transformation of the immortalized nontumorigenic human mesothelial cell line MeT-5A (33). Caspase-1 activation promotes the secretion of IL-1β and requires the assembly of high-molecular-weight complexes known as inflammasomes (34). The Nalp3 inflammasome, the best characterized of these complexes, is activated by a wide variety of stimuli, including exogenous pathogen-associated molecular patterns (PAMP) and endogenous damage-associated molecular patterns [DAMP (34, 35)]. Asbestos induces cell necrosis, causing the release of high-mobility group protein B1 [HMGB1 (36)], a typical DAMP and a key mediator of inflammation (37). This, in turn, can trigger activation of Nalp3 inflammasome and subsequent IL-1β secretion, a process that is enhanced by asbestos-induced production of ROS (38, 39). In one study, Nalp3-deficient mice exhibited decreased pulmonary pathology in response to silica and asbestos exposure compared with wild-type controls (38).

In addition, upon asbestos exposure, HMGB1 is released by reactive macrophages and other inflammatory cells, as well as by human mesothelial cells (HMC; 36). HMGB1 is a critical regulator in the initiation of asbestos-mediated inflammation leading to the release of TNF-α and subsequent NF-κB signaling (36). TNF-α is a central mediator in asbestos-, silica- and bleomycin-induced models of pulmonary fibrogenesis (40). In one study, asbestos-exposed TNF-α receptor knockout mice did not develop the fibroproliferative lesions found in asbestos-exposed wild-type mice (41). TNF-α–induced NF-κB signaling was shown to be the critical link between inflammation and carcinogenesis in multiple cancer models, including mesothelioma (31). Asbestos-mediated TNF-α signaling induces the activation of NF-κB–dependent mechanisms, thus promoting the survival of HMCs after asbestos exposure (31). This allows HMCs with accumulated asbestos-induced genetic damage to survive, divide, and propagate genetic aberrations in premalignant cells that can give rise to a malignant clone.

![Figure 1. Working hypothesis for mesothelioma carcinogenesis.](https://example.com/image.png)
In healthy cells, HMGB1 is found primarily in the nucleus, where it stabilizes chromatin and plays multiple roles in DNA transcription, replication, and recombination. During programmed necrosis, HMGB1 translocates from the nucleus to the cytosol and the extracellular space, where it binds several proinflammatory molecules and triggers the inflammatory responses that distinguish this type of cell death from apoptosis. These findings provide mechanistic links between asbestos-induced cell death, chronic inflammation, and mesothelioma. Secreted HMGB1 stimulates RAGE, TLR2, and TLR4 (the 3 main HMGB1 receptors) expressed on neighboring inflammatory cells such as macrophages and induces the release of several inflammatory cytokines, including TNF-α and IL-1β. In addition, HMGB1 enhances the activity of NF-κB, which promotes tumor formation, progression, and metastasis (42). An investigation into the targeting of extracellular HMGB1 as a novel strategy for mesothelioma prevention and/or therapy is currently underway (43). As illustrated in Fig. 1, we hypothesize that HMGB1 functions as a master switch that initiates a series of inflammatory responses leading to malignant transformation of asbestos- or erionite-damaged HMCs.

Clinical–Translational Advances

Molecular therapies

Although increased expression of EGFR has been noted in human mesothelioma, a phase II clinical trial of the EGFR signaling inhibitor gefitinib yielded disappointing results (44). Because RTKs are frequently activated in mesothelioma, investigators tested the possible benefits of small-molecule RTK inhibitors, including erlotinib and imatinib (Fig. 2). However, the results of such studies to date have not been promising (45, 46). The accumulation of cytoplasmic β-catenin, a downstream component of the Wnt signaling pathway, in the majority of human mesotheliomas indicates that Wnt signaling is abnormally activated (47). Moreover, the dishevelled proteins (also downstream of Wnt) are often overexpressed in mesothelioma, and siRNA knockdown of dishevelled proteins was shown to suppress mesothelioma growth (48). These data suggest that agents that target components of the Wnt signaling pathway could benefit mesothelioma patients. The inverse correlation between VEGF serum levels and mesothelioma patient survival (49) suggests that VEGF signaling contributes to mesothelioma. However, a phase II clinical trial of the humanized anti-VEGF monoclonal antibody bevacizumab plus erlotinib (Fig. 2) in mesothelioma patients yielded no clinical benefits (50). PI3K, AKT, and the downstream mTOR are often found to be activated in mesothelioma, and inhibition of mTOR using rapamycin enhances the apoptosis of mesothelioma cells in vitro (22), which suggests that mTOR may serve as a target for mesothelioma therapies (Fig. 2). The NF-κB signaling pathway is critical for the pathogenesis of mesothelioma. Therapies aimed at inhibiting NF-κB activity, such as ranpirnase and bortezomib, may benefit a small subset (10%) of patients (51–53).

Molecular therapies that target aspects of tumor immunity may also have a significant impact on the course of mesothelioma because the altered microenvironment will affect the ability of the immune system to mount antitumor responses. Sterman and colleagues (54) led several clinical trials examining the effects of intrapleural delivery of type I...
IFN-encoded adenoviruses. These trials showed that high local concentrations of IFN-α or IFN-β were well tolerated and induced strong cellular and humoral antitumor immune responses, leading to tumor cell death. Some patients with mesothelioma experienced prolonged survival.

In summary, molecular therapies have not affected the average survival of mesothelioma patients, although in several trials 5% to 10% of the patients responded and experienced prolonged survival. Investigators in clinical trials normally look at averages, and therefore no significant benefit can be detected for any therapy when the benefit occurs in only a small fraction of patients. Thus, the challenge ahead of us is to identify the subset of patients who will respond to a given type of therapy.

**Targeting asbestos-induced inflammation to prevent or treat mesothelioma**

Chronic inflammation has been associated with an increased risk of developing numerous types of cancer. In this regard, daily treatment with aspirin for ≥5 years was shown to reduce tumor burden in several common malignancies (35), and results from animal experiments support a beneficial role for anti-inflammatory therapies in mesothelioma (56). Thus, we hypothesize that prolonged aspirin treatment may help reduce the incidence of mesothelioma and other asbestos-related malignancies among high-risk cohorts with either a lengthy history of exposure and/or genetic predisposition.

On the basis of recent findings, it is tempting to speculate that HMGB1 and the Nalp3 inflammasome act as critical initiators of chronic inflammation in asbestos- and erionite-exposed individuals, with the secretion of IL-1β and TNF-α acting as the key downstream driving force. Therefore, HMGB1, Nalp3, TNF-α, and IL-1β can all serve as potential targets for inhibitors of asbestos-induced inflammation leading to mesothelioma. Indeed, Hamada and colleagues (57) showed that bronchoalveolar lavage fluid from patients with idiopathic pulmonary fibrosis exhibited elevated levels of HMGB1 and that treatment with an anti-HMGB1 antibody prevented bleomycin-induced lung fibrosis in mice. In addition to mesothelioma, several solid tumors, including melanoma, prostate, pancreatic, breast, and gastrointestinal cancers (42), display elevated levels of HMGB1; therefore, therapies that seek to block HMGB1 signaling would likely prove effective in other cancer types as well as in mesothelioma.

Treatment with an IL-1 receptor antagonist can protect mice from developing fibrosis upon exposure to bleomycin or silica (58). In a murine model of bleomycin- or silica-induced pulmonary fibrosis, infusion with the human recombinant soluble TNF receptor sTNFR-β was effective not only in preventing the development of pulmonary fibrosis but also in the treatment of established fibrosis (40). Similar results were observed with the use of anti-TNF-α antibodies (59). Specific U.S. Food and Drug Administration–approved reagents that inhibit these molecules are available. Anakinra, an IL-1 receptor antagonist, is used in therapies for patients with autoimmune diseases and gout.

Infliximab, a chimeric human–mouse anti-TNF-α, and etanercept, a soluble TNF receptor fusion protein, have both been used to treat patients with rheumatoid arthritis and other diseases, including plaque psoriasis and ankylosing spondylitis. Glyburide, the most widely used sulfonylurea drug for type 2 diabetes in the United States, inhibits the Nalp3 inflammasome (60). Specific molecules that target the activity of HMGB1 are anti-HMGB1 and anti-RAGE antibodies, recombinant HMG Box A, and ethyl pyruvate, an inhibitor of HMGB1 secretion (ref. 42; Fig. 2).

Early detection of mesothelioma is associated with improved clinical outcomes (1). The finding of significantly higher serum levels of HMGB1 in asbestos-exposed individuals compared with cohorts of smokers with histologically proved bronchial inflammation and dysplasia (36) suggests that HMGB1 may be a potential marker of exposure to carcinogenic mineral fibers. Moreover, soluble mesothelin-related peptide (SMRP) and osteopontin were proposed to be candidate markers for the early detection of mesothelioma (i.e., before the appearance of clinical symptoms).

**Conclusions**

Because the latency period from initial asbestos or erionite exposure to disease progression is often decades long (1), novel therapies that prevent or delay carcinogenesis in exposed individuals could lead to a substantial decrease in mesothelioma mortality. In light of our recent increased understanding that asbestos carcinogenesis is linked to chronic inflammation, we can design multiple strategies to target inflammation in asbestos- and erionite-exposed individuals. Clinical and translational research focusing on such strategies has the potential to reduce the impact of the carcinogenic effect of asbestos and erionite exposure. Moreover, genetic testing for BAP1 mutations in exposed cohorts should help us identify genetically susceptible individuals who have the highest risk of developing mesothelioma (10). These individuals could be targeted for early detection, for example, by monitoring HMGB1, SMRP, or other biomarkers. Strategies that seek to prevent carcinogenesis in asbestos/erionite-exposed, high-risk individuals would have the most wide-reaching impact on the incidence of this deadly cancer.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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